SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED

Synopsis

The research work leading to the degree of the

Doctor of philosophy

In

Zoology

Entitled

"Acute Toxic Effect of Neonicotinoid Insecticides (Nitenpyram) On Freshwater Female Crab *Barytelphusa guerini*".

By

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(Research Scholar)

Under the Guidance of

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"Acute Toxic Effect of Neonicotinoid Insecticides (Nitenpyram) On Freshwater Female Crab *Barytelphusa guerini".*

1) Proposed Problem:

Nitenpyram is a chemical frequently used as an insecticide in agriculture and veterinary medicine. The compound is an insect neurotoxin belonging to the class of Neonicotinoids which works by blocking neural signaling of the central nervous system. It does so by binding irreversibly to the nicotinic acetylcholine receptor causing a stop of the flow of ions in the postsynaptic membrane of neurons leading to paralysis and death. Nitenpyram has seen extensive use in targeted, insecticide applications. In 1989, the compound's first documented commercial use was in 1995 under the name "Bestguard" as an agricultural insecticide *Yamamoto, I.; Casida, J.E (1999).* Later, Nitenpyram was expanded for use as a flea treatment by the Novartis company under the trade name "Capstar", with a subsequent FDA approval for non-food producing animals in October 2000. The current producer of Nitenpyram itself is the **Sumitomo** chemical company. Nitenpyram continues to be used commercially, though data from market surveys indicate a significant decrease in the global usage compared to other insecticides or Neonicotinoids.

2) Introduction:

Since their discovery in the late 1980s, Neonicotinoid pesticides have become the most widely used class of insecticides worldwide, with large-scale applications ranging from plant protection (crops, vegetables, fruits), veterinary products, and biocides to invertebrate pest control in fish farming. Neonicotinoid and the phenyl-pyrazole fipronil are insecticides with systemic properties. Their physicochemical characteristics, mainly assessed in terms of their octanol water partition coefficient (Kow) and dissociation constant (pKa), enable their entrance into plant tissues and their translocation to all its parts . Regardless of the manner of application and route of entry to the plant, they translocate throughout all plant tissues making them toxic to any insects (and potentially other organisms) that feed upon the plant. This protects the plant from direct damage by herbivorous (mainly sap feeding) insects and indirectly from damage by plant viruses that are transmitted by insects. The discovery of imidacloprid by Shinzo Kagabu, and its subsequent market introduction in 1991, started the era of the Neonicotinoid class of insecticides.

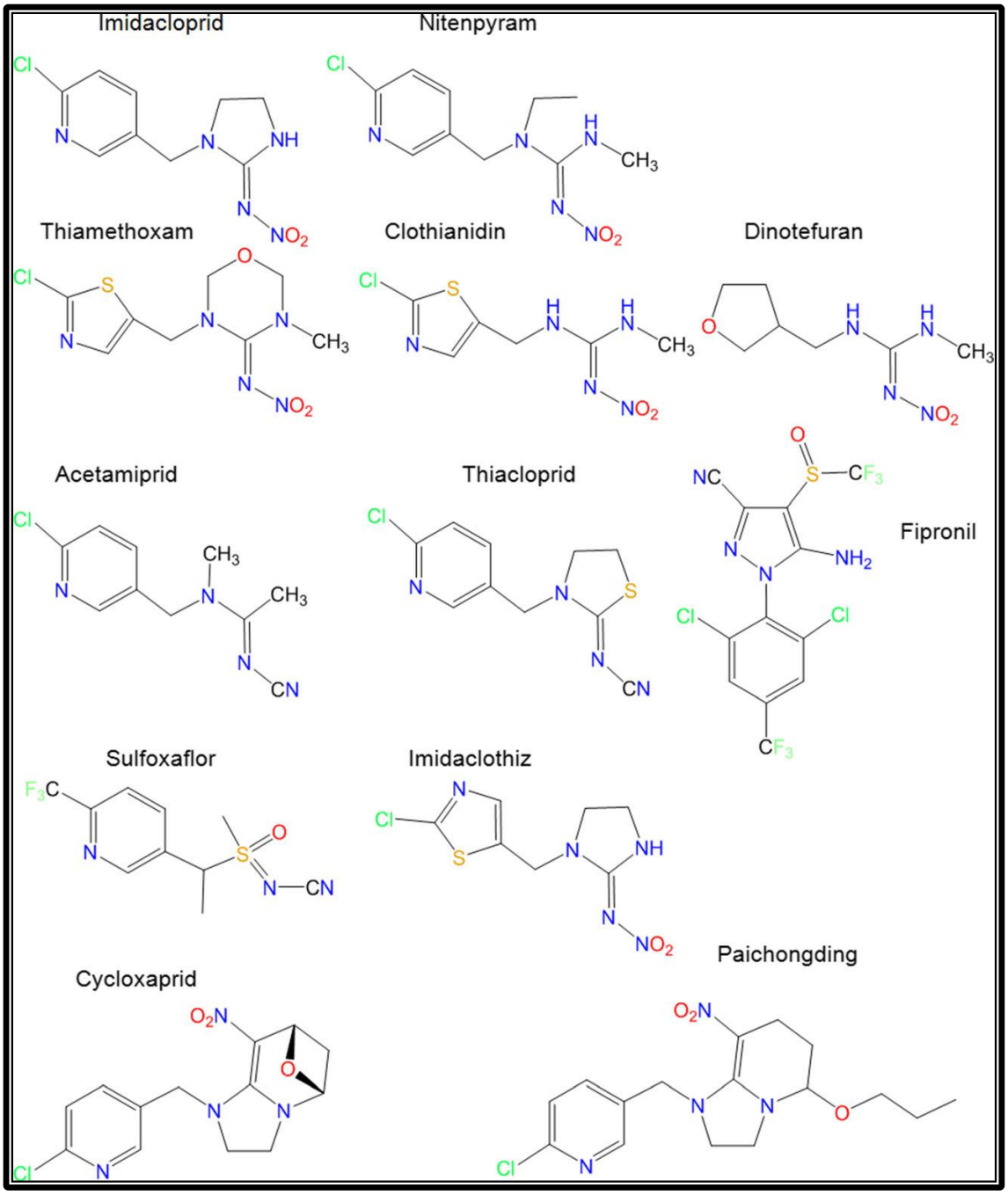
Imidacloprid was followed in 1999 by thiamethoxam and clothianidin, which is a metabolite of thiamethoxam (Meredith et al. 2002). Over the following two decades, Neonicotinoids have become the most widely used insecticides of the five major chemical classes (the others being organophosphates, carbonates, phenyl-pyrazoles, and pyrethroids) on the global market (Jeschke et al. 2011; Casida and Durkin 2013). The French company Rhône-Poulenc Agro (now Bayer Crop Science) discovered and developed fipronil between 1985 and 1987, reaching the market in 1993 (Tomlin 2000). It is noteworthy that substances belonging to the phenyl-pyrazole family have in principal herbicidal effects, whereas fipronil is a potent insecticide. By the 1980s, many pest insects had developed resistance to the organophosphates, carbamates, and pyrethroids then on the market (Alyokhin et al. 2008). Set against this background of increased resistance to existing insecticides, the Neonicotinoid and fipronil were presented as having several key attributes that led to their rapid adoption in both agricultural and urban environments. These included the following: lower binding

efficiencies to vertebrate compared to invertebrate receptors, indicating selective toxicity to arthropods, high persistence, systemic nature, versatility in application (especially as seed treatments), high water solubility, and assumed lower impacts on fish and other vertebrates. The binding sites of Neonicotinoid to nicotinic acetylcholine receptors (nAChRs) and fipronil to γ-aminobutiric acid (GABA) receptors in the nervous systems of vertebrates are different from those in insects. In general, vertebrates have lower numbers of nicotinic receptors with high affinity to neonicotinoids, which is why neonicotinoids generally show a priori higher toxicity to invertebrates than vertebrates (including human, e.g., Tomizawa and Casida 2005). Similarly, the binding of fipronil to insect GABA receptors is tighter than that observed for vertebrate receptors (Narahashi et al. 2010). This, combined with the frequent use on neonicotinoids and fipronil in seed/soil treatments rather than sprays, is supposed to make them comparatively safe for agricultural workers. This is in contrast to some of the alternatives that they have replaced, such as organophosphates and carbamates (Marrs 1993).

Neonicotinoids and fipronil are also relatively persistent, offering the potential for long-term crop protection activity. The half-lives of these compounds in aerobic soil conditions can vary widely, but are measured in months or longer (e.g., 148–6,931 days for clothianidin; USEPA 2003). Extensive information about the physicochemical characteristics of neonicotinoids and fipronil can be found in Bonmatin et al. (2014), together with information about their environmental fate. Arguably, however, it is the systemic nature of these insecticides that has made them so successful. Irrespective of their mode of application, neonicotinoids become distributed throughout the plant, including the apices of new vegetation growth, making them particularly effective against sucking pests, both above ground and below.

Although it is not a neonicotinoid, fipronil also acts systemically mainly when it is co-formulated with polymers to increase its systemic activity. Neonicotinoids and fipronil belong to a wide family of substances jointly referred to as the “systemic insecticides” due to their systemic properties, some carbamate and organophosphorus substances, however, can also act systemically (Sanchez-Bayo et al. 2013). Neonicotinoid and fipronil should theoretically not target organisms lacking nervous systems, such as protists, prokaryotes, and plants. Very little research has been done on these non-target organisms and the ecosystem functions they are responsible for. Nevertheless, some studies have revealed negative effects: for example, a negative effect of fipronil on soil microorganisms was suggested as a possible cause for the slower (ca. four-fold) degradation of this pesticide at high vs. low application in Australian soils (Ying and Kookana 2006). Seven separate neonicotinoid compounds are available commercially worldwide (Jeschke et al. 2011).

These are imidacloprid and thiacloprid (developed by Bayer CropScience), clothianidin (Bayer CropScience and Sumitomo), thiamethoxam (Syngenta), acetamiprid (Nippon Soda), nitenpyram (Sumitomo), and dinotefuran (Mitsui Chemicals). An eighth compound, sulfoxaflor (Zhu et al. 2010), has recently come onto the market in China and the USA and has been reviewed by the European Food Safety Authority (EFSA) for approval in the European Union (EFSA 2014). In China, new Neonicotinoid compounds are being developed and tested (e.g., guadipyr and huanyanglin), and are nearing market release . Some of these novel Neonicotinoid are the cisneonicotinoids, which are isomers of Neonicotinoid in which the nitro or cyano group are in the cis, rather than trans, orientation. It is well known that trans and cis isomers can differ markedly in their toxicity.



Global growth in the insecticide market:

In 1990, the global insecticide market was dominated by carbamates, organophosphates, and pyrethroids. By 2008, one quarter of the insecticide market was neonicotinoid (rising to 27 % in Casida and Durkin 2013), and nearly 30 % was neonicotinoid and fipronil combined, with the other classes correspondingly reduced (Jeschke et al. 2011). In the same year, imidacloprid became the world's largest selling insecticide, and second largest selling pesticide (glyphosate was the largest; Pollack 2011) with registered uses for over 140 crops in 120 countries (Jeschke et al. 2011). Neonicotinoids are no win widespread use for a wide variety of crops worldwide.

3) Review of Literature:

1) **Lennard Pisa et al.** An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems Environ Sci Pollut Res Int. 2021; 28(10): 11749–11797.Published online 2017 Nov 9. doi: 10.1007/s11356-017-0341-3

New information on the lethal and sublethal effects of neonicotinoids and fipronil on organisms is presented in this review, complementing the previous Worldwide Integrated Assessment (WIA) in 2015. The high toxicity of these systemic insecticides to invertebrates has been confirmed and expanded to include more species and compounds. Most of the recent research has focused on bees and the sublethal and ecological impacts

2) **US Environmental Protection Agency (USEPA) (2003a)** Factsh - Clothianidin. 19 pp

Neonicotinoid insecticides have become one of the most widely used insecticides over the past two decades. Recent studies have shown considerable risk of Neonicotinoid to beneficial insects, however, their health risks to mammals are still under debate. Limited empirical mammalian toxicity information for Neonicotinoid inherently presents challenges to environmental health practitioners performing health hazard and risk assessment.

3) **Liu GY, Ju XL, Cheng J (2010)** Selectivity of imidacloprid for fruit flyversus rat nicotinic acetylcholine receptors by molecular modeling. J Mol Model 16:993–1002

For better understanding of the mechanisms of selective binding of the representative nicotinic acetylcholine receptor (nAChR) agonist neonicotinoid Imidacloprid (IMI), three-dimensional models of fruit fly alpha 1 beta 2 and rat alpha 4beta 2 nAChRs were generated by homology modeling, using the crystal structure of the acetylcholine-binding protein (AChBP) of Lymnaea stagnalis and the nAChR of mus musculus as the templates, respectively. The calculated docking energies were in agreement with the experimental data and the putative binding sites were also consistent with the results from labeling and mutagenesis experiments. Furthermore, the mechanisms of Imidacloprid selectively acting on fruit fly versus rat nAChRs were discussed.

4) **Aajoud A, Ravanel P, Tissut M (2003)** Fipronil metabolism and dissipation in a simplified aquatic ecosystem. J Agric Food Chem51:1347–1352

Several phenylpyrazole derivatives are selective inhibitors of chloride channel activities in insects. In this chemical family, fipronil is a powerful insecticide now widely used for several purposes. The dissipation of this molecule in a simplified aquatic ecosystem has been studied for 3 months, using 14C-labeled fipronil. The main features of the complex process leading to fipronil transformation in this system were the following. The fipronil aqueous solution was submitted to two chemical transformations: the photodependent desulfuration of the side chain bound to the 4-position of the heterocyclic ring and the chemical hydrolysis of the nitrile function bound to the 3-position. Fipronil, rapidly transferred from the water .solution to the organic matter, was protected from the previously mentioned chemical transformations but evolved to give two main metabolites, which were either reduced or oxidized in the side chain on the 4-position.

5) **Casida JE (2011)** Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. J Agric Food Chem 59:2923–2931

Neonicotinoid are one of the three principal insecticide chemotypes. The seven major commercial Neonicotinoid are readily biodegraded by metabolic attack at their N-heterocyclylmethyl moiety, heterocyclic or acyclic spacer, and N-nitroimine, nitro methylene, or N-cyanoimine tip. Phase I metabolism is largely dependent on microsomal CYP450 isozymes with situ selectivity in hydroxylation, desaturation, dealkylation, sulfoxidation, and nitro reduction. Cytosolic aldehyde oxidase is a nitroreductase for some Neonicotinoid. Phase II metabolism involves methylation, acetylation, and formation of glucuronide, glucoside, amino acid, and sulfate- and glutathione-derived conjugates. Some neonicotinoids act as proinsecticides with metabolism to more potent nicotinic agonists. Pest resistance is more commonly due to synergist-reversible CYP450 detoxification than to nAChR mutants or variants. Metabolites in some cases contribute to mammalian hepatotoxicity and carcinogenesis and in others to enhanced plant vigor and stress shields. These relationships explain much of neonicotinoid comparative toxicology and provide the basis for continued and improved safety and effectiveness of this chemo type.

6) **Miyagi S, Komaki I, Ozoe Y (2006)** Identification of a high-affinity binding site for dinotefuran in the nerve cord of the American cockroach. Pest Manag Sci 62:293–298

The binding of the neonicotinoid insecticide dinotefuran to insect nicotinic acetylcholine receptors (nAChRs) was examined by a centrifugation method using the nerve cord membranes of American cockroaches and [3H]dinotefuran (78 Ci mmol-1). The Kd and Bmax values of [3H]dinotefuran binding were estimated to be 13.7 nM and 14.8 fmol 40 microg-1 protein respectively by Scatchard analysis. Epibatidine, an nAChR agonist, showed a rather lower affinity to the dinotefuran binding site (IC50=991 nM) than dinotefuran (IC50=5.02 nM). Imidacloprid and nereistoxin displayed lower potencies than dinotefuran but higher potencies than epibatidine.

7) **Tomizawa M, Casida JE (2005)** Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu Rev Pharmacol Toxicol 45: 247–268

The neonicotinoids, the newest major class of insecticides, have outstanding potency and systemic action for crop protection against piercing-sucking pests, and they are highly effective for flea control on cats and dogs. Their common names are acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam. They generally have low toxicity to mammals (acute and chronic), birds, and fish. Biotransformations involve some activation reactions but largely detoxification mechanisms. In contrast to nicotine, epibatidine, and other ammonium or iminium nicotinoids, which are mostly protonated at physiological pH, the neonicotinoids are not protonated and have an electronegative nitro or cyano pharmacophore.

4) Objective of Research:

Neonicotinoid have been the most commonly used insecticides since the early 1990s. Despite their efficacy in improving crop protection and management, these agrochemicals have gained recent attention for their negative impacts on non-target species such as honeybees and aquatic invertebrates. In recent years, Neonicotinoid have been detected in rivers and streams across the world. Determining and predicting the exposure potential of Neonicotinoid in surface water requires a thorough understanding of their fate and transport mechanisms. Therefore, our objective was to provide a comprehensive review of Neonicotinoid with a focus on their fate and transport mechanisms to and within surface waters and their occurrence in waterways throughout the world. A better understanding of fate and transport mechanisms will enable researchers to accurately predict occurrence and persistence of insecticides entering surface waters and potential exposure to non-target organisms in agricultural intensive regions. This review has direct implications on how Neonicotinoid are monitored and degraded in aquatic ecosystems. Further, an improved understanding of the fate and transport of Neonicotinoid aide natural resource practitioners in the development and implementation of effective best management practices to reduce the potential impact and exposure of Neonicotinoid in waterways and aquatic ecosystems.

1) To study the regulation of Insecticide
2) To study Residual Problem of Synthetic Chemical which are used as Systemic Pesticides.
3) To Study the Effect of Insecticide in related to Metabolism and Physiological aspect on Aquatic Invertebrates.
4) To study effect on Biomolecules.

5) Methodology:

1) **Toxic Evaluation:** LC_{50} value method used Followed By Finney (1964)
2) **Behavioural Study:** Quick Screening procedure for monitoring effects of Environmental Pollution (Janardhan Reddy 1988)
3) **Heart Beat Rate:** By Crab Ringer Solution.
4) **Oxygen Consumption:** Wrinkler Idometric Method (Welsh and Smith, 1953)
5) **Estimation of Protein:** Lowry's Method (Lowry, O H, 1951)
6) **Estimation of Glycogen:** Anthrone Reagent Method (Selfers, et al, 1956)
7) **Estimation of Lipid:** Methanol : Chloroform Method (Floch, J. et al., 1956)
8) **Estimation of Amino Acid:** Ninhydrin Method (Rosen H., 1957)
9) **Estimation of Blood Sugar :** Somogyi- Shaffer Hartmann Method (Schaffer, P.A. 1933)
10) **Estimation of DNA :** Diphenylamine Method (Rosen, H., 1957)

6) Importance of Study:

1) **To Study Environmental Effects**

Chemical pesticides are responsible for polluting our environment while being used in the field. Although their effects are short lived, they may begin causing hazardous environmental and health problems if they persist in the environment. When chemical pesticides are sprayed on the crops, they may spread to regions beyond the intended area.

2) **Effect on Crops and Soil and Life**

Pesticides degrade the quality of soil and further the quality of the food and yield. Chemical pesticides deplete the nutritional value of the food and contaminate it. When we eat any food such as apple, lettuce, potatoes or strawberries, these pesticides enter our body and may lead to many health issues. While these chemical pesticides are developed to kill organisms found on the crop, these are also harmful to other living things.

3) **Biotic Poisoning:**

Pesticides have also been shown to disrupt the balance of an ecosystem. In many situations, when a pesticide is used, it also kills non-pest organisms. This can drastically alter the natural balance of the ecosystem. By removing non-pest organisms, the environment can be changed to favor the pest. In addition to causing harm to wildlife, pesticides that travel from their original location are known to cause harm to humans. Human exposure to pesticides has caused poisonings, the development of cancer and the deaths of between 20,000 and 40,000 people worldwide each year.

4) **Bio Accumulation:**

Another major problem associated with pesticide use is bioaccumulation and biological magnification. Bioaccumulation is when a substance builds up in the body because the body does not have the proper mechanisms to remove it. Many synthetic pesticides are not able to be broken down. Once they enter the body of an organism, they are permanently stored in the body tissue.

7) Proposed Work Plan with Chapter:

1) **Chapter I: Introduction**

The great solvent power of water has been making the creation of absolutely pure water a theoretical rather than a practical Goal. Water contamination by pesticides and Insecticides is serious problem. The awareness and option is needing full to protect the Nature and Balancing the Ecological equilibrium. All Collective Current and Previous information collected in this Chapter.

2) **Chapter-II: Material and Method:**

The effect of Nitenpyram, Insecticide causes various changes in Metabolic as well as Physiological activity in aquatic invertebrate female crab Barytelphusa guerini.

Acute toxicity accelerates deposition and make residual problem in tissues they make rushes in protein, Carbohydrates, Lipid, Amino acid and Nucleic Acid.

This Chapter Follows the Scientific protocol as

1**) Toxic Evaluation**: LC 50 value method used Followed by Finney (1964)

2**) Behavioural Study**: Quick Screening procedure for monitoring effects of Environmental Pollution (Janardhan Reddy 1988)

3) **Heart Beat Rate:** By Crab Ringer Solution.

4) **Oxygen Consumption**: Wrinkler Idometric Method (Welsh and Smith, 1953)

5**) Estimation of Protein**: Lowry's Method (Lowry, O H, 1951)

6**) Estimation of Glycogen**: Anthrone Reagent Method (Selfers, et al, 1956)

7**) Estimation of Lipid**: Methanol: Chloroform Method (Floch, J. et al., 1956)

8) **Estimation of Amino Acid**: Ninhydrin Method (Rosen H., 1957)

9) **Estimation of Blood Sugar:** Somogyi- Shaffer Hartmann Method (Schaffer, P.A. 1933)

10**) Estimation of DNA:** Diphenylamine Method (Rosen, H., 1957)

3) **Chapter-III: Toxicity Evaluation:**

The contamination of environmental pollution is becoming serious problem because of perfuse use of pesticides. Chemicals in agriculture, Industry and Heavy metals.

The poison is worst and requires safety measures in the place where industrial discharge carries the toxicant in proportion. The toxic evaluation of Nitenpyram to the fresh water Female crab *Barytelphusa guerini* was detected precise dosages level, LC_{50} value method.

4) **Chapter-IV: Behavioural Study:**

The Behaviour represent an integrated response of the organism in the functioning of any one of the body systems results in behavioural change. It would form one of the quick screening procedures for monitoring the effect of environmental pollution view of this it would be appropriate to review certain behavioural abnormalities in crab treated with Nitenpyram.

5) **Chapter-V: Result and Discussion:**

The acute toxic effect on various metabolite variations might be in all possibility due to difference in the biochemical construction of the tissues and Blood in respect to toxicant.

In this chapter effect of Nitenpyram, titled observation and tabulates by various tools of Statistical method. In this added all observations and results with tables and Graphs in relation to topic sited as

1) Heart Beat Rate: By Crab Ringer Solution.
2) Oxygen Consumption: Wrinkler Idometric Method (Welsh and Smith, 1953)
3) Estimation of Protein: Lowry's Method (Lowry, O H, 1951)
4) Estimation of Glycogen: Anthrone Reagent Method (Selfers, et al, 1956)
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7) Estimation of Blood Sugar: Somogyi- Shaffer Hartmann Method (Schaffer, P.A. 1933)
8) Estimation of DNA: Diphenylamine Method (Rosen, H., 1957)

6) **Chapter: VI: Summary and Conclusion:**

The result and observations of the research work is summarized in summary and Conclusion.

7) **Chapter-VII: Bibliography:**

References used and sited in literature are listed in the Bibliography.

8) Year wise Plan:

1) **I- year Work:**

In first six month collect the protocol and literature from journals, Reference Book. Secondly six month period count the LC_{50} value and Monitor the Behavioural observations.

2) **II- Year Work:**

In first six month calculate biochemical experiments Heart Beat rate and Oxygen consumption. Secondly six months Biochemical Experiments Estimation of Protein and Glycogen.

3) **III- Year Work:**

In first six month calculate biochemical experiments Estimation of Lipid and Amino Acid. Secondly six months Biochemical Experiments Estimation of Amino Acid and DNA.

9) Expected Outcome

The research work having following outcomes

1) Neonicotinoids are high deposition potential causes degrade molecular building in invertebrates, We got deposition ratio.
2) It causes decline rate of Metabolism like Respiration, effect of Heart beat rate etc.
3) It shows effect on Natural source of Protein.
4) Residual problem cause cancer possibility to human kind as circulating contaminated food.

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